IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First named inventor: ARENDT Thomas

Application No: 10/576,142 Group Art Unit: 1649

Filing Date: December 6, 2006 Examiner: Stacey MacFarlane

DECLARATION PURSUANT TO 37 C.F.R. 1.132

I, Thomas Arendt hereby declare:

1. I received a M.D. in Medicine and a D.Sc. in Neuroscience, from the University of Leipzig, Germany. I have been professor of neuroanatomy and head of the department of neuroanatomy at the faculty of medicine of the University of Leipzig since 1996. I have worked in the field of brain research for more than 25 years.

I am one of the inventors of U.S. patent application no. 10/576,142 "Quick test for the Diagnosis of Alzheimer´s Disease".

2. I have read and understood the Office Action dated May 15, 2009. I understand that the claims were rejected for lack of enablement, because the Examiner has concluded that a skilled artisan cannot practice the invention without first making a substantial inventive contribution in that a skilled artisan would have to demonstrate that the method wherein the stimulation index of at least 10 is indicative of a diagnosis of Alzheimer´s.

3. In order to demonstrate that the claimed invention is already enabled by the originally filed application wherein the stimulation index of at least 10 for the mitogenic surface marker CD69 upon stimulation with the mitogenic factor PHA is indicative of a diagnosis of Alzheimer´s disease the following study regarding the stimulation index is provided, wherein the samples were prepared according to the example described in the specification:

4. Material and Methods

4.1 Participants

The present study included 21 patients with AD ("AD study group") and 17 age-matched controls ("Control study group") without mental impairment. The diagnosis of probable AD according to ICD-10 research criteria (including a neuropsychiatric evaluation based on the Mini-Mental State Examination) and the collection of blood samples (5 ml each) were carried out by the Department of Psychiatry, University of Leipzig.

4.2 Assay Methods

Blood samples were taken by venous puncture into 5ml tubes (heparin/Na-citrate, SARSTEDT AG & Co.). Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll-Paque PLUS (GE Healthcare) as described in the product guidelines. Isolated cells were stimulated with Phytohaemagglutinine (PHA-L, 12µg/ml, Sigma-Aldrich). The non-stimulated reference sample contained an equal volume of complete media. Samples were incubated at 37°C with 5% CO_2 for 4 hours, subsequently mixed and fixed with FACS-Lysing solution (BD Biosciences). Staining of cells was performed with the following antibody-cocktails (BD Biosciences): A – IgG1-FITC/IgG1-PE/CD3- PerCP; B – CD4-FITC/CD69-PE/CD3-PerCP, C – CD19-FITC/CD69-PE/CD45-PerCP. Samples were analyzed using a calibrated FacsCalibur Flow cytometer and CellQuest software (BD Biosciences). A total of $1x10^4$ gated events were collected for each data file.

4.3 Data Analysis and Statistical Evaluation

The calculation of the Stimulation index (SI) was based on raw data obtained by flow cytometry. SI is defined by the ratio of the percentages of CD69-positive cells after and before stimulation after the determination of a cut-off point between CD69-positive and CD69-negative cells on the basis of a control sample (isotype control).

The raw data and the calculated activation index is shown in Table 1a-1c wherein the numbers of CD69 positive (CD69+) and CD69 negative (CD69-) events of CD45+ cells which represents all the lymphocytes in non-stimulated probe and after the stimulation are shown for each subject.

The stimulation index (SI) was calculated according to the following formula:

$$SI = \frac{\dfrac{n[CD69+]\ PHA\text{-}stim.}{(n[CD69+]\ PHA\text{-}stim. + n[CD69-]\ PHA\text{-}stim.)}}{\dfrac{n[CD69+]\ non\text{-}stim.}{(n[CD69+]\ non\text{-}stim. + n[CD69-]\ non\text{-}stim.)}}$$

Tables 1a-1c also show the respective MMSE (mini-mental state examination) values. MMSE is commonly used to estimate the severity of cognitive impairment in an individual: Generally, a score over 27 is considered as normal; 20-26 indicate some cognitive impairment; 10-19 moderate to severe cognitive impairment, and below 10 very severe cognitive impairment.

5. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that theses statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

November 10, 2009

Prof. Dr. Thomas Arendt

Patient	1	2	3	4	5	6	7	8	9
Study Group	AD	AD	AD	AD	AD	AD	AD	AD	AD
MMSE	6	25	18	22	11	24	23	14	3
Age	89	83	83	73	69	82	82	81	75
number of CD69+/CD69- events of CD45+ cells									
non-stimulated [CD69+]	224	283	528	205	531	329	678	488	293
non-stimulated [CD69-]	10046	8796	8957	10079	11577	6908	8937	9256	9740
PHA-stimulated [CD69+]	1381	776	523	597	334	173	1100	132	991
PHA-stimulated [CD69-]	1541	615	390	1075	318	169	369	121	2045
Stimulation index	21,67	17,9	10,29	17,91	11,68	11,13	10,62	10,42	11,18

Patient-ID	10	11	12	13	14	15	16	17	18
Study Group	AD	AD	AD	AD	AD	AD	AD	AD	AD
MMSE	5	13	17	9	2	7	28	27	17
Age	73	57	76	78	80	78	81	69	59
number of CD69+/CD69- events of CD45+ cells									
non-stimulated [CD69+]	341	626	178	111	216	629	138	142	32
non-stimulated [CD69-]	9706	9023	9824	10316	9101	10031	9583	9802	9505
PHA-stimulated [CD69+]	1604	332	283	972	176	909	213	1021	51
PHA-stimulated [CD69-]	894	252	275	2279	279	1237	568	1016	93
Stimulation index	18,92	8,76	28,5	28,09	16,68	7,18	19,21	35,1	105,55

Table 1a

Patient	19	20	21
Study Group	AD	AD	AD
MMSE	14	14	23
Age	90	89	60
number of CD69+/CD69- events of CD45+ cells			
non-stimulated [CD69+]	269	727	49
non-stimulated [CD69-]	10173	9217	10822
PHA-stimulated [CD69+]	3128	804	1261
PHA-stimulated [CD69-]	2486	110	5848
Stimulation index	21,63	12,03	39,35

Table 1b

Patient	C1	C2	C3	C4	C5	C6	C7	C8	C9
Study Group	Control	Control	Control	Control	Control	Control	Control	Control	Control
MMSE	29	28	29	29	28	28	28	29	30
Age	86	85	88	85	70	93	91	84	85
number of CD69+/CD69- events of CD45+ cells									
non-stimulated [CD69+]	2220	279	866	699	324	180	386	1359	378
non-stimulated [CD69-]	7508	458	8317	4480	9579	9458	9147	7522	7845
PHA-stimulated [CD69+]	1687	221	292	434	834	346	1944	736	879
PHA-stimulated [CD69-]	275	116	111	186	449	288	3397	1128	1087
Stimulation index	3,77	1,73	7,68	5,19	19,87	29,22	8,99	2,58	9,73

Patient	C10	C11	C12	C13	C14	C15	C16	C17
Study Group	Control	Control	Control	Control	Control	Control	Control	Control
MMSE	28	29	29	30	27	29	29	28
Age	85	91	91	92	85	85	85	77
number of CD69+/CD69- events of CD45+ cells								
non-stimulated [CD69+]	955	2158	944	390	321	523	569	796
non-stimulated [CD69-]	8051	2901	8940	9456	9491	8467	6987	9371
PHA-stimulated [CD69+]	389	757	296	221	1135	411	204	1360
PHA-stimulated [CD69-]	202	1015	102	59	1895	357	125	1508
Stimulation index	6,21	1,00	7,79	19,93	11,45	9,2	8,23	6,06

Table 1c